# Mirror Peripheral Neuropathy and Unilateral Chronic Neuropathic Pain

*Insights from Asymmetric Neurological Patterns in Leprosy*

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# Mirror peripheral neuropathy and unilateral chronic neuropathic pain: insights from asymmetric neurological patterns in leprosy

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Abstract

Leprosy-related multiple mononeuropathy offers a pattern of impairment where neuropathy with and without neuropathic pain (NeP) are present in the same individual, thus allowing to investigate peripheral sensory and innervation in both conditions. This cross-sectional study collected data on clinical and neurological examination, pain-assessment questionnaires, quantitative sensory test, and intraepidermal nerve fiber density (IENFD) of patients with leprosy and divided the cohort into two groups: with (P+) and without NeP (P-). Further, we assessed mirror body areas in the same NeP individuals with bilateral neuropathy also presenting unilateral NeP. Pain-free patients having unilateral neuropathy were controls. A total of 37 P+ patients and 22 P- patients were evaluated. Limb areas with NeP had signs of C-fiber dysfunction and hyperesthesia on QST compared with limb areas having neuropathy without NeP. Skin denervation was found in all leprosy patients. Comparisons of limbs with and without neuropathy, and with and without NeP revealed that higher heat pain thresholds (HPT) were associated with neuropathic pain areas, whereas less altered HPT was correlated with higher fiber density. Furthermore, a relationship was found between time of leprosy treatment termination and more intense neuropathy, expressed by HPT increasing 0.03 ºC each month. As expected, interindividual comparisons failed to show differences in IENFD and subepidermal plexus areas between patients P+ and P- ($p=0.2980$, $p = 0.9044$; respectively). Higher HPT and lower mechanical detection threshold were related to

NeP. The present study pointed out the relevance of intra-individual comparisons including mirror areas when assessing local changes in peripheral NeP.

Keywords: leprosy, skin biopsy, neuropathic pain, intraepidermal nerve fibers, quantitative sensory testing

## Introduction

Neuropathic pain (NeP) affects about 6.9%–10% of the general population [52], with even higher prevalence among those with specific disorders such as cancer (28.2%) [2], spinal cord lesions (55.6%) [51], and infectious diseases including HIV sensory neuropathy (90.0%) [40] and leprosy (56.1%) [44]. Despite recent advances and treatment options being included in treatment guidelines [34], patients with NeP may remain refractory to first-, second-, and third-line treatments. Up to 40% of patients with NeP reportedly present with pharmaco-resistant NeP [38,15], sparking a very intense quest for biological [22], imaging [21], and psychophysics biomarkers of subtypes of NeP based on putative symptom-mechanism correlations, instead of an etiology-based approach [18,1]. It is established that patients within a certain NeP etiology have more differences than similarities concerning their symptoms and mechanisms of pain generation, leading to several attempts to group patients according to clinical phenotypes, usually based on pain symptoms or psychophysical profiles [44,1,54]. While this strategy has been promising and correlated accurately with response to treatment in some instances [5], it has not completely resolved the quest for patient characterization and improvement in patient selection for pharmacological treatment [35,16]. Specifically, most studies compared patients with and without NeP among those with the same disease. This is a pragmatic and clinically oriented methodology; however, in such an approach, individual characteristics potentially related to the occurrence of NeP, such as

intensity of skin denervation and sensory thresholds, are mixed with personal and individual traits such as catastrophizing, mood, sleep, and cognitive states that will influence the report of pain [25]. Moreover, several studies analyzed data such as skin samples (for intraepidermal nerve fiber density measurement) in body areas not necessarily affected by pain [41,49,58]. This approach may not identify important local factors associated with NeP, but that were diluted among other higher-order inter-individual characteristics affecting pain perception [39].

To overcome these issues, we took a novel approach and chose to study patients with cured leprosy, a condition where:

i. Mononeuritis multiplex (MNM) is the main clinical presentation, so that, neuropathy affects some nerve trunks, while spares others. This allows the contralateral mirror area to serve as proper control for peripheral phenomena.

ii. Neuropathy affects mainly nerve trunks located on the limbs, allowing side-to-side comparisons that are not possible in polyneuropathies or when spinal cord is also affected.

iii. Limbs can be primarily compared, rather than individuals. In patients with NeP (P+), this allows comparing limbs with neuropathy (n+), presenting with (p+) or without (p-) NeP. This approach circumvents the inter-personal variables present when studying the role of local factors in pain occurrence. Additionally, this approach allows for studying pain-free control individuals (P-) who have limbs with (n+) and without neuropathy (n-) to assess the abnormalities associated with neuropathy without pain.

Therefore, we chose patients with chronic, cured leprosy having MNM to primarily compare the limbs of patients with and without NeP, as a window to the contribution of local sensory and pathological findings in NeP development.

## Methods

### *Standard Protocol Approvals, Registrations, and Patient Consents*

This study was approved by the Institutional Ethics Committee of Hospital das Clínicas da Universidade de São Paulo, São Paulo, Brazil (approval number 217.288). All participants provided written informed consent.

### *Participants*

In an outpatient setting in the dermatology clinical division of the Hospital das Clínicas da Universidade de São Paulo, 360 adult patients with leprosy were routinely followed-up. Leprosy was diagnosed in patients with two or more of the following: skin lesions typical for leprosy, thickened peripheral nerves, and acid-fast bacilli on slit skin smears [56]. Inclusion criteria were: completion of polychemotherapy (PCT) for ≥6 months, not under leprosy reaction, not currently taking therapy for leprosy reactions (thalidomide and prednisone), and not presenting neuritis. Reaction type 1 (reversal reaction) was diagnosed when a patient had erythema and edema of skin lesions or neuritis. Reaction type 2 (erythema nodosum) was diagnosed when a patient had tender subcutaneous skin lesions, accompanied or not by neuritis, iritis, arthritis, orchitis, dactylitis, lymphadenopathy, edema, or fever [29]. Neuritis was diagnosed as new neural function loss, with sensory or motor impairment [29]. Patients with comorbidities, other peripheral neuropathy etiologies, or difficulties in understanding the testing procedure and those aged <18 years were excluded. The included participants had NeP (P+) and two mirror limbs (either forearm-forearm (n=18), thigh-thigh (n=7), or lower leg-lower leg (n=34)) both affected by neuropathy, one associated with NeP (n+p+) and the other only presenting deficits but not pain (n+p-). Those without chronic pain comprised the

control group (P-). Control group also had two mirror limbs where one was affected by neuropathy (without pain (n+p-)) and the other was without signs of neuropathy (n-p-), based on undetected thermal roller test temperatures (TRT; Somedic®, Hörby, Sweden). Warm and cold TRT was used to screen large body areas of non-painful thermal sensory deficits and consisted of two manual rollers at predetermined temperatures of 40 °C and 25 °C at a speed of 2 cm/s. Upper or lower mirror limb areas were compared with a third control area located in the dorsum midline, one of the warmest areas of the body and is known to be spared from leprosy neuropathy [9]. According to the aforementioned criteria, 114 patients were prospectively invited to participate and were screened by TRT. Fifty-nine participants, who fulfilled inclusion and had no exclusion criteria, underwent full assessment. Patients were diagnosed with definite NeP if the pain distribution was neuroanatomically plausible and if clinical examination confirmed that negative or positive sensory signs (i.e., hypo/anesthesia, hypo/analgesia, hyperalgesia, or allodynia) were confined to the innervation territory of the affected nervous structure (i.e., pain of neuropathic characteristics [positive Douleur Neuropathique 4 questionnaire]), and if they had leprosy (disease-causing neuropathy) and clear signs of neuropathy on examination [17].

***Study Design***

This cross-sectional study collected demographic data, information on clinical and neurological examination, pain questionnaires, quantitative sensory testing (QST), and intraepidermal nerve fiber density (IENFD) determination during a single visit in patients with leprosy (P+ and P-).

***Data Collection***

*Demographics*

Information on sex, age, skin color, occupation, educational background, socioeconomic status, type of leprosy, and duration since the end of the full PCT course were recorded.

*Pain-assessment questionnaires*

Patients filled in five questionnaires. The short form of the Brief Pain Inventory (BPI) that measures pain intensity, determines pain location, treatments or medications in use for pain relief, and the interference that pain causes in patients' lives on a scale of 0–10 [13]. The Douleur Neuropathique 4 questionnaire (DN-4), a screening tool for NeP, consisting of 10 items including questions and clinical signs (scored one if present and 0 if not; total score ≥4 points) suggests NeP [48]. The short-form McGill Pain Questionnaire (sf-MPQ) consists of characterization of pain using 15 descriptors grouped into three: eight sensitive, five affective, and two evaluative, with binary score: present or absent [14]. The Neuropathic Pain Symptoms Inventory (NPSI) is an instrument to detect different symptoms and dimensions of NeP. There are 10 descriptors scored between 0–10 with a final score of 0–100 [4]. The hospital anxiety and depression scale (HAD) consists of 14 items, seven for assessing anxiety and seven for depression; each item is scored between 0–3, with a maximum score of 21 points for each of the scales [3].

*Laboratory assessments*

The following laboratory data were collected: urea, creatinine, electrolytes, fasting blood glucose, glycated hemoglobin, thyroid stimulating hormone (TSH), free T4, aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), albumin, vitamin B12, calcium, serological reaction for syphilis, HIV serology, and complete blood count.

***Standardized Neurological Examination (SNE)***

The neurological examination included a systematic evaluation of tendon reflexes, superficial and proprioceptive sensory (ataxia was scored based on the Nobile-Orazio score) [37], pain, trophism, and vasomotor abnormalities as previously described [28].

Involvement of myelinated sensory nerve fibers was assumed in the following situations: loss of tactile/vibration sensation in the skin on any part of the limbs; decrease/absence of reflexes; >1 score for ataxia or severe alteration in the detection of finger position. Involvement of thin myelinated or non-myelinated sensory nerve fibers was assumed in the following situations: alteration of pinprick sensory in any part of the limb; presence of trophic, vasomotor, or sudomotor alterations; and visual analogue scale (VAS) score of >40 mm [28].

***Psychophysics***

QST was performed in P+ (n+p+), P+ (n+p-), P- (n+p-), and P- (n-p-) limbs of the patients. Mechanical detection threshold (MecDT) and mechanical pain threshold (MPT), defined as the lowest pressure that generated touch and pain sensation, respectively, were measured using calibrated (0.008–300 g) von Frey monofilaments (NC17775, Bioseb®, France) [8]. While patients kept their eyes closed, nylon monofilaments were applied perpendicular to the skin surface for approximately 1 s, until the beginning of deformation. Ascending and descending sequencies of pressure were used according to the forced choice method. The average of three runs were used as results for mechanical detection and pain threshold determinations. During these tests, patients had no visual contact with the examined region [8].

Thermal detection and thermal pain thresholds were measured using a VSA-3000/TSA-II 2001 (Medoc®, Ramat Yshai, Israel). To obtain the detection thresholds for warm and cold,

patients were instructed to press a button on perceiving a change in temperature. When the stimulus caused the onset of pain, the values were recorded as the heat pain threshold (HPT) and cold pain threshold (CPT). In all instances, the "method of limits" was used. Cooling and heating were performed with ramped stimuli (1 °C/s) from a baseline temperature of 32 °C. The Peltier thermode (20 × 35 mm) was placed in contact with the tested areas at a baseline temperature of 32 °C and gently removed after stimulation. Temperatures did not exceed 50 °C for heat stimuli and 0 °C for cold stimuli. The values of three consecutive measurements were computed. All thermal thresholds were expressed as absolute temperature values [36]. Suprathreshold heat pain stimuli (heat hyperalgesia) were also analyzed, and the temperature increased at a linear rate of 2 °C/s from a baseline temperature of 32 °C and was maintained for 2 s at two different temperature values (46 °C and 48 °C). The perceived pain intensity was recorded on a VAS and graded from 0 to 100 mm. The infrathreshold cold stimuli (cold thermal hyperalgesia) comprised a decrease in temperature from 32 ºC to 10 ºC and 5 °C, at 2 °C/s. The mean of two VAS scores during suprathreshold heat stimuli (46 °C and 48 °C) and cold infrathreshold stimuli (10 °C and 5 °C) were calculated. Vibration detection thresholds (Hertz [Hz]) were determined via a Rydel-Seiffer tuning fork from 128 Hz scale 8/8 in tibia crest and lateral malleolus [36].

***Skin Punch Biopsy***

A 5-mm diameter skin punch biopsy was obtained from P+ (n+p+), P+ (n+p-), P- (n+p-), and P- (n-p-) limbs under local anesthesia. The location of the biopsy was determined by the most painful area or areas of thermal deficit in cases where no pain existed. Control neuropathy and mirror area biopsies were collected at the corresponding body area contralaterally. The skin punch biopsy sample was fixed in freshly prepared 4% paraformaldehyde at 4 °C for 2 h and further processed for visualization of epidermal nerve fibers on 40-µm cryosections.

Sections were immunoreacted with antibodies against the pan-axonal marker protein-gene product 9.5 (PGP9.5; Ultraclone®, Wellow, UK, 1:800), using goat anti-rabbit IgG labeled with Cy3 (Cy3; Jackson®, PA, USA, 1:100). Protein-gene product 9.5-positive nerve fibers were visually quantified on coded slides using a fluorescent microscope by two investigators, blinded to the participant's diagnosis, according to published criteria [23]. To quantify myelinated fibers and nodal length, double immunofluorescence staining was performed using anti-MBP (GeneTex, 1:500) and anti-Caspr antibodies (Abcam, Cambridge, 1:100) [7]. The subepidermal plexus was quantified using a density threshold for PGP 9.5 brightness, and the area was expressed as a percentage of the whole subepidermal area analyzed (200 x 50 μm), in every third optical field along the epidermal length, the mean of the obtained values was used for further comparisons, as previously described [53].

***Statistical Analyses***

P+ patients had their sample size calculated by the G*Power3.1® software [12] using the effect size d=0.67 [33],[39] value obtained for the most important variable in the study, IENFD, calculated from previous studies [33], which resulted in a needed sample of 33 individuals with the Wilcoxon paired test, alpha of 0.05, and power of 0.95. It was decided to include 37 patients to prevent possible losses. P- patients were 22 patients matched for age and gender. SNE, QST, and neuropathology findings in P+ (n+p+), P+ (n+p-), P- (n+p-), and P- (n-p-) limbs were compared using the Chi-square test or paired means comparisons (Wilcoxon test). Individuals with (P+) and without (P-) NeP were compared on demographic profile, leprosy characteristics, and pain questionnaire responses, using Chi-square or Mann-Whitney tests. Intra-individual modeling analysis (multivariable analysis) included each patient being assessed in two mirror body areas, with 118 limbs considered independently for analysis. QST significant variables for pain limbs were assessed to investigate relationships in these

limbs using generalized additive models for location, scale, and shape (GAMLSS). The response variable used for modeling was the basal temperature variation, for example for HPT: QST heat pain threshold minus 32 °C. This response variable acts as a censored variable as after a given temperature, the experiment must cease. Hence, censorship on the right occurs when heat pain reaches 18 °C (the thermode reaches 50 °C, and then 50 °C minus 32 °C = 18 °C). Modeling strategy: the GAMLSS package was used in the software R® (version Rx64 3.3.2) and RStudio® (version 1.0.136), with censored distribution for the response variable. As a strategy, the initial modeling was performed with the distribution of the response variable as normal censored on the right. Significant variables were selected using the StepGAIC algorithm. Distributions with better accommodation to waste and better adjustments were chosen. Variables not significant at the 10% level remained and were removed one by one. The model for the HPT used a normal distribution with censorship on the right, with the identity link function for μ (significance level of 10%). The MecDT model applied log to the response variable, the adjusted model was normal linear, and the response had a log-normal distribution (significance level of 10%). Pain predictors: in this analysis, the objective was to verify which of the selected explanatory variables were more capable to explain whether an individual presented pain or not. As we obtained data from 59 individuals and more than 20 variables of interest, we made a pre-selection of variables to include in a mathematical modeling analysis for pain prediction. Logistic regressions and successive Least Absolute Shrinkage and Selection Operator (LASSO) variable selection for regression were performed one by one with each predictor of interest and the descriptive levels obtained. Subsequently, we assessed the same estimates by generalized linear model (GLM) and by GAMLSS. After the mathematical modeling, the pain predictive capacity of the model was measured by a receiver operating characteristic (ROC) Curve.

***Data Availability:*** Any data not published within the article will be available upon reasonable request.

## Results

### *Patient Characteristics*

Patients with NeP consisted of 23 women (62.2%) and 14 men (37.8%), with a mean age of 48.0 ± 13.6 years. In the pain-free group, there were 9 women (40.9%) and 13 men (59.1%), with a mean age of 49.9 ± 16.3 years ($p>0.05$) (eTable 1). The distribution of clinical forms of leprosy and time since treatment end and assessment were not different between the two groups ($p>0.4$) (eTable 2).

### *Pain-Assessment Questionnaires*

All items in the BPI, DN-4, sf-MPQ, and HAD questionnaires differed significantly between P+ and P- groups (eTable 3). In patients with NeP, higher scores were obtained for the descriptors: tingling, burning, and pins and needles from NPSI (Figure 1). All NPSI sensory descriptors compared between P+ and P- groups were significantly different ($p <0.01$), being higher in tingling and burning. The five pain dimensions were compared between groups: superficial component of spontaneous pain ($p = 0.0001$), deep component of spontaneous pain ($p = 0.0001$), spontaneous paroxysmal pain ($p = 0.0001$), evoked pain ($p = 0.0001$), and paresthesia/dysesthesia ($p = 0.0001$). The total NPSI score also differed between groups ($p = 0.0001$; eTable 3).

### *Laboratory Assessments*

The laboratory tests revealed four patients with altered fasting blood glucose, but normal glycated hemoglobin and no history of diabetes; as it may correspond to non-fasting conditions, they were referred for further investigation. One patient had B12 deficiency and underwent replacement therapy.

***Intra-individual Limb Characterization According to Standardized Neurological Examination, Psychophysics, and Quantitative Evaluation of IENFD, Subepidermal Plexus, and Myelinated Dermal Fibers***

*SNE*

Most limbs examined from patients with leprosy presented clinical evidence of neuropathy and signs of involvement of myelinated, slightly myelinated, and unmyelinated nerve fibers. SNE results by limb are represented in eTable 4.

*Psychophysics*

HPT was higher in P+ (n+p+) (47.5 ± 3.4 ºC) than in P+ (n+p-) limbs (45.9 ± 3.6 ºC), p = 0.010, while MecDT was lower (1.5 ± 3.6 g) in P+ (n+p+) than in P+ (n+p-) limbs (5.8 ± 29.6 g) (p = 0.047). The pairwise analyses between P- (n+p-) and its mirror contralateral limbs P- (n-p-) also showed a difference for the MecDT (p = 0.043) and HPT (p = 0.023). Comparisons including the four limb groups showed a significant difference for MecDT (p <0.0001), HPT (p = 0.0289), and cold hyperalgesia (p = 0. 0331) (Table 1).

*IENFD, subepidermal plexus, and myelinated dermal fibers*

Patients with leprosy had severe loss of skin innervation in all distal leg sampled skin areas; the mean IENFD ranged between 2.1–3.2 fibers/mm (<5% percentile) compared to normative data [42]. There were no group differences between the four patient limbs groups with

respect to IENFD (Figure 2). Comparing these values of IENFD with normative data for immunofluorescence at the distal lower limb [42], among the 37 P+ patients, in (n+p+) limbs, 23 had reduced IENFD (below the 5$^{th}$ percentile). In (n+p-) limbs, 22 patients had reduced IENFD. In 22 pain-free patients, 15 (n+p-) limbs had showed reduced IENFD, while such abnormalities occurred in 14 (n-p-) limbs.

There were no differences between P+ (n+p+), P+ (n+p-), P- (n+p-), and P- (n-p-) limbs in the subepidermal plexus area (Figure 2).

Most skin samples (66%) from patients with leprosy did not contain myelinated dermal fibers. The average number of bundles of myelinated fibers per section ranged between 1.5–3.2 bundles. There was no difference between limbs regarding the number of bundles of myelinated fibers (Figure 2). Mean Ranvier node length were 6.8±2.5 mm for P+ (n+p+) limbs; 6.3±2.5 mm for P+ (n+p-); 7.9±1.3 mm for P- (n+p-); and 7.4±3.3 mm for P- (n-p-), with no significant difference between groups (Figure 2).

***Intra-individual Limbs Modelling Analysis between QST Variables, Pain Presence, IENFD, Leprosy Type, and Time of Completion of Treatment - Multivariable Analysis***

The QST variables HPT and MecDT for pain limbs were modeled, as they showed significant differences in pairwise comparisons. The estimates are presented in Table 2 and Table 3. (eAppendix 1, available as supplemental digital content at http://links.lww.com/PAIN/B701) (eFigure 1). In HPT modelling, P+ (n+p-) limbs had a higher HPT of 2.3 °C than P- (n-p-) limbs, and P+ (n+p+) limbs had a record of HPT of 3.45˚C higher than the P- (n-p-) limbs. Further, totally denervated areas (IENFD = 0/mm) showed an increase in the estimated HPT of 4.03˚C, while the partially innervated samples (IENFD = 1–5 fibers / mm) showed an estimated increase in HPT of 1.54˚C compared to reference limbs (IENFD >5 fibers/mm). Time and worsening of neuropathy were found related. With each additional month between

the end of PCT treatment and the current assessment, HPT increased by 0.03 °C, and the paucibacillary and dimorphic leprosy types showed a lower HPT compared to the multibacillary form (-2.66 ˚C and -4.25 ˚C, p = 0.002, respectively). In MecDT modelling, P+ (n+p-) limbs had, on average, an increase of 150.9% in MecDT response (significance level of 10 %) compared to P- (n-p-) limbs; P+ (n+p+) limbs had an average increase of 331.4% in the MecDT response compared to P- (n-p-) limbs. Limbs with IENFD >0 and ≤ 5 fibers/mm had a mean MecDT increase of 368.6%. Each month between the end of PCT and the present evaluation implied an average increase of 1.2% in the MecDT. Each additional unit (1/10) in the burning score in the NPSI increased by 14.75% the MecDT. The increase in one unit of evoked pain score in NPSI decreased 14.94% in MecDT, while the increase in the number of previous type 1 reactions decreased 46.97% in MecDT. (eAppendix 2) (eFigure 2, available as supplemental digital content at http://links.lww.com/PAIN/B701).

***Pain Predictors***

GAMLSS results are expressed in Table 4, with differences in painful limbs for spontaneous pain and paresthesia/dysesthesia from the NPSI (items 1, 11, 12), and heat hyperalgesia (eAppendix 3) (eFigure 3, available as supplemental digital content at http://links.lww.com/PAIN/B701). ROC analysis was used to assess the ability of the model to predict pain. The area under the curve (AUC) of the model was 0.9865, indicating a high capacity to predict presence/absence of pain in a patient, in possession of the variables related to superficial spontaneous pain and paresthesia/dysesthesia from the NPSI (items 1, 11, 12), and heat hyperalgesia (Figure 3). The optimum cutoff point, at which the sensitivity (0.9545) and specificity (0.9189) of the model was maximized was 0.4506783. This means that, if the predicted value of the model is less than the cutoff-point, the patient must be classified as

having pain, if more, as without pain. The model was able to correctly classify 55 of the 59 patients.

**Discussion**

Signs of myelinated, thinly myelinated, and unmyelinated damaged nerve fibers were present in most limbs, suggesting predominance of combined large and small-fiber neuropathy in patients treated for ≥6 months and considered cured. Limbs with NeP showed higher HPTs and mechanical hyperesthesia compared to limbs without NeP. IENFD assessment revealed severe skin denervation in patients. However, no group differences were found when limbs were compared for IENFD, subepidermal plexus area, number of myelin bundles, and Ranvier node length. Impaired heat pain was associated with NeP and lower nerve fiber density in limb groups. The pain predictor analysis model showed that superficial spontaneous pain, paresthesia/dysesthesia symptoms, and heat hyperalgesia could predict the presence of pain with good sensitivity and specificity in this sample. Specifically, the present study showed higher HPT impairment association with lower IENFD, where totally denervated limbs and partially innervated limbs showed higher HPT compared with more innervated limbs, with comparisons performed among limbs in an intraindividual basis. Previous studies designed in an inter-individual basis showed similar HPT findings. Karlsson et. al. found that 8 of 16 (50%) studies corroborated the association of higher HPT temperature with lower IENFD in distal symmetric polyneuropathy of different etiologies [24]. From 17 studies that assessed IENFD and MecDT, 1 of 7 (14%) detected higher MecDT with lower IENFD showing similar direction as the present investigation. Among the analyzed variables in this review, WDT and HPT showed stronger association with IENFD as compared with cold thresholds [24]. These discoveries could be because IENFD mostly reflect heat-conducting C fibers compared to cold-conducting Aδ fibers. Another study

reported reduction in HPT associated with NeP during oxaliplatin treatment in an inter-individual analysis [27]. A further study showed an anatomical reduction of cutaneous innervation density in the upper dermis and epidermis of painful postherpetic neuralgia skin, which is significantly and positively correlated with loss of thermal sensory function, also as an inter-individual comparison [47]. Heat hypoalgesia in areas with NeP was also described in Guillain-Barré syndrome [32], and with several grouped neuropathies [55].

Leprosy burdens public health systems, especially in developing countries. Globally, 202.185 new cases were detected in 2019, with a rate of 25.9 per million population [57]. Nerve injury affects nerve trunks, superficial and cutaneous nerves, and the nerve endings of the peripheral nervous system, impairing sensory, motor, and autonomic functions [9]. Leprosy neuropathy is long-standing with acute and subacute outbreaks occurring during its insidious course, starting in young patients [43,50]. A large proportion of patients have presented chronic NeP, mostly after pharmacological treatment/cure, which poses an intense negative impact on their quality of life and functionality [31].

Previous studies using QST showed extensive signs of neuropathy in leprosy. Lund et al., performed tests at fixed temperatures (50°C and 4°C) and evaluated mechanical stimuli with monofilaments and painful stimuli with weighted needles [31]. Eleven out of the 17 assessed had sensory loss in all modalities, and none had signs of hyperalgesia or allodynia [31]. We found preservation of vibration detection threshold in our QST battery, which is in line with previous reports such as Haroun et al., who reported vibration perception to be preserved in most patients. In their study, the QST methodology used could not distinguish between groups of patients with and without pain in an inter-individual comparison [19,20]. Recent studies [6,10] have reported on the occurrence of alterations on QST over the painless mirror limbs in instances of unilateral painful neuropathy/complex regional pain syndrome unrelated to leprosy. However, similar findings have also been reported in central neuropathic pain

after unilateral strokes [26] and could be related to limitations of QST methodology employed in these studies, which included reactions-time-dependent approaches to sensory perception threshold determination. While the exact reasons explaining the findings of bilateral sensory deficits in patients with unilateral diseases remain to be determined, their existence suggest that we potentially underestimated the precise side-to side differences between pain and painless neuropathy sites reported here. In fact, due to our study design, it was possible to compare limbs with neuropathic pain to limbs with painless neuropathy, and also to limbs without overt neuropathy.

A previous study with skin biopsies from skin leprosy lesions and contralateral areas without lesions demonstrated a decrease in intraepidermal fibers in areas with and without lesions. However, the contralateral subepidermal plexus was preserved as in the controls [11]. In subsequent studies, specimens of contralateral regions showed a decrease in the intraepidermal fiber density in untreated patients [46]. Lund et al. found reduced innervation in 16 out of 17 patients with leprosy having chronic NeP using skin and nerve biopsy; additionally, they performed an IENFD comparison between patients with mononeuropathy and polyneuropathy with the latter showing a greater IENFD reduction than the former [31]. In these studies, specimens were from areas with skin lesions or regions outside the pain area. Herein, skin biopsies were collected in areas with neuropathy and NeP and in the contralateral areas with neuropathy, and the biopsy site selection was guided by the presence of pain. Significant intraepidermal and subepidermal denervation was found in patients P+ (n+p+) and in P+ (n+p-), and in patients P- in P- (n+p-) and in P- (n-p-) by sensory screening using TRT, similar to previous studies that showed intense subclinical denervation in patients with leprosy [31]. However, there was no difference in IENFD and subepidermal plexus areas independent of the presence/absence of NeP. Facer et al. identified that increased thermal thresholds in the leprosy affected skin correlated with decreased subepidermal nerve

fibers [11]. However, they found no correlation between IENFD and the degree of impairment in thermal thresholds, since fiber rarefaction was homogeneously intense in all patients, regardless of the degree of sensory deficit. These studies [11,46] involved untreated patients, and the site of skin biopsy was not guided by the presence of pain as in the present study. Here, mathematical modeling revealed a relationship between HPT and IENFD. Further, in this cross-sectional study, HPT and MecDT changes were related to NeP and may have future prognostic and screening value, should these findings be replicated in larger cohorts.

We found a relationship between the duration since PCT termination and severity of neuropathy, expressed by HPT increase. This may be accounted for by the neurotoxic effects of drug treatments over peripheral nerves [30], presenting coasting effects [30] and building up for some time after treatment discontinuation. However, another explanation is that fragments of destroyed bacterial capsule contributing to neuropathy. It has long been demonstrated that fragments and debris of mycobacterium capsule induce phenotypical changes in peripheral nerves, also affecting Schwann cells and potentially leading to neuronal degeneration even in the absence of live and viable bacteria [45]. This alternative hypothesis could explain the occurrence of *de novo* NeP after treatment completion, its maintenance, and may also contribute to reactions occurring after bacterial cure.

This cross-sectional study was limited because of the sample population with severe neuropathy and significant intraepidermal and subepidermal denervation, instead of recently diagnosed leprosy neuropathy where sensory damage includes an early loss of pain and temperature perception depending on temperature distribution [9]. While our aim was to study cured patients who represent the largest burden of chronic NeP associated with leprosy, this inevitably led to a sample with a high neuropathy burden, which may have blunted

potential group differences due to ceiling effects. The patients presented with neuropathic pain after leprosy diagnosis was made and before PCT was started, but the specific duration of neuropathic pain was not available. Future studies with larger samples will probably include a wider profile of patients with diverse pattern of neuropathy and will further add to this topic.

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**Competing interests:**

The authors have declared that there are no competing interests.

**Supplemental video content**

A video abstract associated with this article can be found at http://links.lww.com/PAIN/B702.

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**Tables**

**Table 1.** QST comparison between limbs: P+(n+p+), P+(n+p-), P-(n+p-), and P-(n-p-)

| QST parameters | [P+(n+p+)] (n=37) | [P+(n+p-)] (n=37) | [P-(n+p-)] (n=22) | [P-(n-p-)] (n=22) | [P+(n+p+)] X [P+(n+p-)] X [P-(n+p-)] X [P-(n-p-)] | [P+(n+p+)] X [P+(n+p-)] | [P-(n+p-)] X [P-(n-p-)] |
|---|---|---|---|---|---|---|---|
| | mean±SD (min-max) | mean±SD (min-max) | mean±SD (min-max) | mean±SD (min-max) | | | |
| Mechanical detection threshold (g) | 1.5±3.6 (0.008-15) | 5.8±29.6 (0.008-180) | 1.8±3.8 (0.008-17) | 5.3±21.3 (0.008-100) | <0.0001£ | 0.047§ | 0.043§ |
| Cold detection threshold (ºC) | 20.9±9.1 (0-30.5) | 24.7±5.6 (0-31.2) | 21.6±9.5 (0-29.7) | 22.9±8.2 (0-31.9) | 0.4290 | | |
| Warm detection | 42.0±5.7 (33.2-50) | 39.8±5.2 (33.3-50) | 39.4±9.4 (4.4-50) | 40.2±6 (32.2-50) | 0.3524 | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| threshold (ºC) | | | | | | | |
| Mechanical pain threshold (g) | 39.6±85.1 (0-300) | 30.0±73.6 (0.07-300) | 94.7±131 (0.04-300) | 79.8±116.2 (0.02-300) | 0.1340 | | |
| Cold pain threshold (ºC) | 8.4±8.5 (0-27.2) | 12.0±8.6 (0-26.4) | 7.6±8.9 (0-25.4) | 10.3±9.5 (0-27.3) | 0.1337 | | |
| Heat pain threshold (ºC) | 47.5±3.4 (38.6-50) | 45.9±3.6 (36.5-50) | 47.8±2.9 (37-50) | 45.6±4.5 (35.4-50) | 0.0289£ | 0.010§ | 0.023§ |
| Mechanical hyperalgesia (/100) | 17.6±15.5 (0-51) | 16.9±13.2 (0-49) | 12.7±15,5 (0-50) | 14.5±18.8 (0-78) | 0.2401 | | |
| Cold hyperalgesia (/100) | 26.2±29.2 (0-89.5) | 27.6±23.6 (0-76) | 12.6±17.7 (0-32) | 15.6±18.7 (0-73) | 0.0331£ | | |
| Heat hyperalgesia (/100) | 29.4±29.5 (0-99) | 29.2±25.2 (0-86) | 14.5±19.7 (0-83) | 19.1±21.2 (0-77.5) | 0.0637 | | |
| Dynamic mechanical allodynia (/100) | 4.1±12.2 (0-62) | 1.1±3.8 (0-20) | 2.6±12.2 (0-57) | 1.8±8.5 (0-40) | 0.1781 | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Temporal summation[α] | 0.9±0.9 (0-3) | 0.9±0.7 (0-3.1) | 0.8±1.1 (0-4.5) | 0.7±0.8 (0-3) | 0.3689 | | |
| Vibration detection threshold (Hz) | 6.8±1.6 (2-8) | 6.7±1.5 (2-8) | 6.5±1.4 (2-8) | 6.6±1.5 (2-8) | 0.5982 | | |

α: adimensional; Hz (hertz); SD: standard deviation; £ statistically significant ANOVA Kruskal-Wallis analysis; § statistically significant contralateral Wilcoxon paired analysis; QST: quantitative sensory testing; [P+(n+p+)]: limbs with neuropathy and pain; [P+(n+p-)]: limbs with neuropathy and without pain; [P-(n+p-)]: limbs with neuropathy; [P-(n-p-)]: non-neuropathy contralateral limbs; $^{o}$C (degrees centigrade); g (grams); /100: 0–100 mm;

**Table 2.** Estimates of the relationship patterns between HPT in the studied limbs, P+ (n+ p+) and P+ (n+ p-), and potentially related variables like pain presence, intraepidermal nerve fiber density, leprosy type, and time between treatment termination and evaluation (in months), according to the normal distribution model with censorship on the right using identity link function for μ

| Variable | Estimate | Inferior limit IC (95%) | Superior limit IC (95%) | P-value |
|---|---|---|---|---|
| P-(n-p-) limbs | ------- | ------- | ------- | ------- |
| P+(n+p-) limbs | 2.302 | 0.403 | 4.202 | 0.019 |
| P+(n+p+) limbs | 3.450 | 1.411 | 5.490 | 0.001 |
| Reference limb | ------- | ------- | ------- | ------- |

| | | | | |
|---|---|---|---|---|
| (IENFD>5 fibers/mm) | | | | |
| Partly innervated limbs (IENFD=1-5 fibers/mm) | 1.544 | -0.462 | 3.551 | 0.134 |
| Fully denervated limbs (IENFD=0/mm) | 4.037 | 1.710 | 6.364 | <0.001 |
| Multibacillary leprosy | ------- | ------- | ------- | ------- |
| Paucibacillary leprosy | -2.656 | -4.325 | -0.987 | 0.002 |
| Dimorphic leprosy | -4.249 | -6.813 | -1.685 | 0.002 |
| Elapsed time between the end of treatment and the present evaluation (in months) | 0.028 | 0.012 | 0.043 | <0.001 |

P+(n+p+): limbs with neuropathy and pain; P+(n+p-): limbs with neuropathy and without pain; IENFD: intraepidermal nerve fiber density; HPT: heat pain threshold.

**Table 3.** Estimates of the relationship patterns between MecDT in the studied limbs, P+ (n+ p+) and P+ (n+ p-), and potentially related variables (presence of pain, intraepidermal fiber density, time between treatment termination and evaluation (in months), symptoms of NPSI, and number of previous type 1 reactions), according to the log-normal distribution model

| Variable | Estimate | Inferior limit IC (95%) | Superior limit IC (95%) | P-value |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| P-(n-p-) limbs | ------- | ------- | ------- | ------- |
| P+(n+p-) limbs | -3.920 | 0.893 | 7.052 | 0.084 |
| P+(n+p+) limbs | 0.920 | 1.315 | 14.146 | 0.017 |
| Reference limbs (IENFD>5 fibers/mm) | ------- | ------- | ------- | ------- |
| Partly innervated limbs (IENFD=1-5 fibers/mm) | 1.461 | 1.567 | 14.015 | 0.007 |
| Fully denervated limbs (IENFD=0/mm) | 0.046 | 0.334 | 3.284 | 0.937 |
| Elapsed time between the end of treatment and the present evaluation (in months) | 0.012 | 1.004 | 1.020 | 0.003 |
| Burning (NPSI) | 0.137 | 1.011 | 1.302 | 0.036 |
| Evoked pain (NPSI) | -0.162 | 0.730 | 0.991 | 0.041 |
| Type 1 previous reaction | -0.634 | 0.270 | 1.046 | 0.070 |

P+(n+p+): limbs with neuropathy and pain; P+(n+p-): limbs with neuropathy and without pain; IENFD: intraepidermal nerve fiber density; NPSI: Neuropathic Pain Symptoms Inventory; MecDT: mechanical detection threshold.

**Table 4.** Estimates of the relationship patterns between variables with statistically significant differences in painful limbs, according to logistic regression

| Variable | | | Pain impact | Inferior limit IC (95%) | Superior limit IC (95%) | P-value (t - GAMLSS) |
|---|---|---|---|---|---|---|
| Burning (Q1 NPSI) Superficial spontaneous pain | | | 2.605 | 1.176 | 5.772 | 0.0219 |
| Pins and needles/ tingling (Q11Q12 NPSI) Paresthesia/dysesthesia | | | 4.060 | 1.326 | 12.428 | 0.0173 |
| Heat hyperalgesia | | | 1.131 | 1.020 | 1.255 | 0.0227 |

NPSI: Neuropathic Pain Symptoms Inventory; GAMLSS: generalized additive models for location, scale, and shape.

**Figure legends**

**Figure 1. (A) Assessment of neuropathic symptoms using NPSI in P+.**

**(B) Assessment of neuropathic symptoms using NPSI in P-.**

NPSI: neuropathic pain symptoms inventory; P+: patients with chronic neuropathic pain; P-: patients without chronic pain.

**Figure 2. Comparison of intraepidermal nerve fibers, subepidermal plexus areas, myelin bundles, and Ranvier node length between P+(n+p+), P+(n+p-), P-(n+p-) and P-(n-p-) limbs.**

[P+(n+p+)]: limbs with neuropathy and pain; [P+(n+p-)]: mirror limbs with neuropathy and without pain; [P-(n+p-)]: limbs with neuropathy; [P-(n-p-)]: non-neuropathic mirror limbs

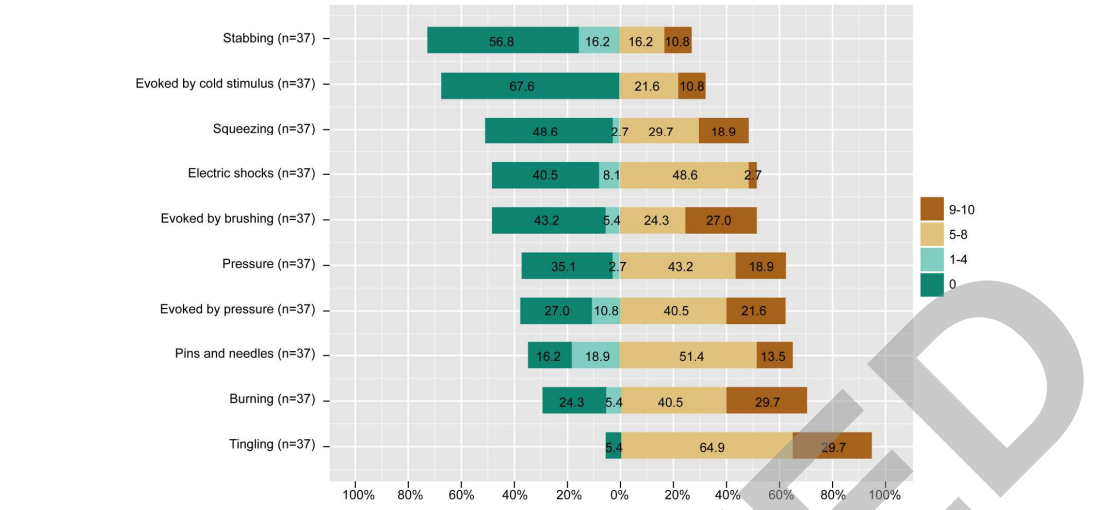
* Size 200×50 mm in each optical field third

--- insufficient data size for Wilcoxon test

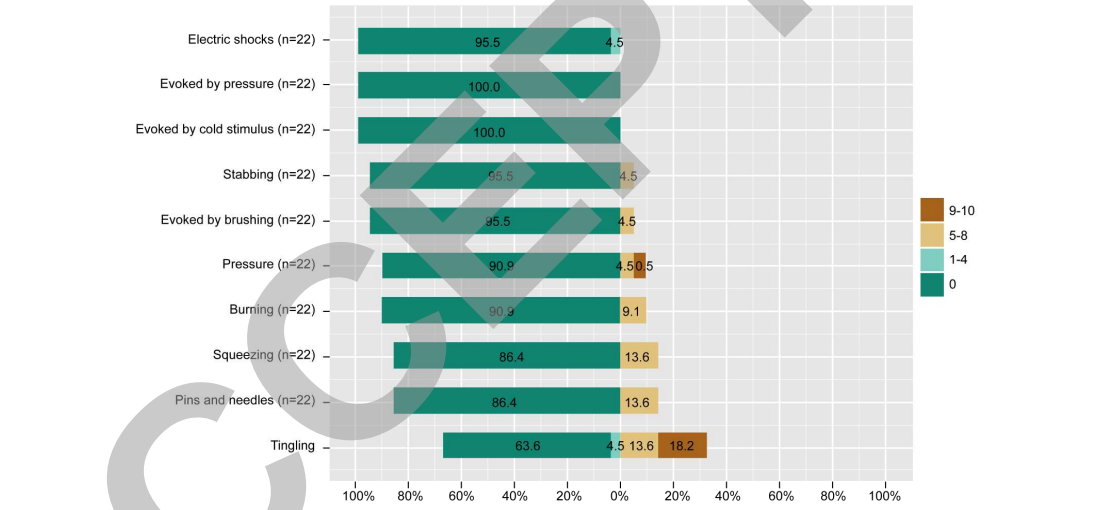
**Figure 3. ROC curve for the pain prediction model.**

ROC curve represents the accuracy performance of the explanatory variables: spontaneous pain from the NPSI, paresthesia/dysesthesia from the NPSI, and heat hyperalgesia from the QST in classifying the patient as having or not having pain. The optimum cutoff point, at which the sensitivity (0.9545) and specificity (0.9189) of the model was maximized was 0.4506783. The area under the curve (AUC) of the model was 0.9865.

AUC: area under the curve NPSI: neuropathic pain symptoms inventory; ROC: receiver operating characteristic




A




B